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PHENOTYPIC CHARACTERIZATION, ESTIMATION OF KERNEL CAROTENOID AND THEIR CORRELATION STUDIES IN MAIZE LANDRACES OF MANIPUR, INDIA

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ABSTRACT

The 42 selected maize landraces of Manipur were analyzed to study genetic variability and correlation for the 8 quantitative traits and a qualitative trait. All the character understudied had genetic variability and some characters have a significant correlation. To confirm does kernel color have the direct relation in the amount of total carotenoid concentration separate correlation studies between total carotenoid (quantitative traits) and kernel color (qualitative trait) was analyzed. The results revealed significant genetic variation among the maize landraces of Manipur for all the characters understudied and there exist a significant positive correlation between 100 kernel weight with plant height, ear height with plant height and growing degree unit to silking with growing degree unit to tasseling whereas negative correlation exists between ear height and total carotenoid. Estimation of total carotenoid concentration was done with the value ranging from 11.48 µg/ g to 41.40 µg/ g and a mean of 19.78 µg/ g. Highest carotenoid concentration was recorded in landraces accession code CAUCH 66. No correlation was found between kernel total carotenoid concentration and kernel color. Total carotenoid and Kernel color correlation studies by both the method of Pearson correlation and Spearman's rank correlation gave the same valid result. The varying degree of total carotenoid comparing to kernel color is due to the involvement of several enzymes other than phytoene synthase which gives yellow color and controls the rate-limiting step in the carotenoid biosynthetic pathway.

KEYWORDS: Manipur, Zea mays L, Carotenoids, Vitamin A, Landraces, Correlation, Kernel Color & Total Carotenoid

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Original Article

INTRODUCTION

Maize (*Zea mays* L.) occupies the third important cereal crop in India. However, in the North Eastern Himalayan region, it is the second most important crop next to rice. Landraces are the cultivar of pre-scientific or primitive agriculture which represents excellent sources of useful genes. Most of the genetic variability is represented within and between landraces maintained by the traditional family farming system Marshall (1977). A wide variety of maize landraces were collected from the Himalayan foot Hills of North East India including Manipur I. A. R. I.(1972), but they did not studied systematically and to improve the important agronomic traits knowledge of the correlations between agronomic traits are essential in plant breeding programs. It helps breeders to choose the most appropriate selection strategy.

Carotenoids are the natural pigments of lipid soluble polyenes which occur widely in plants and serve several functions in plants. A considerable amount of carotenoid compounds which can be converted into Vitamin

A in the human and animal body. They are naturally present in the Maize kernel Wurtzel(2004). Orange and yellow maize are rich in carotenoids that the human body can convert into vitamin A during the metabolic process Harjes et al. (2008). Dietary carotenoids are the essential precursors to Vitamin A and to retinoid compounds needed in animal morphogenesis Bendich and Olson (1989). Apart from Vitamin A property carotenoid also have an antioxidant property which helps the body to protect cells, tissues, and organs against the damaging effect of free radicals, which may contribute to the development disorders such as cancer and heart disease.

Hence the present study was made to characterize and estimate character association of 8 quantitative morphological traits and select the landraces with important agronomic traits. Further estimation of total carotenoid and correlation studies with the maize kernel will assist in understanding the relationship between total carotenoid with kernel color.

MATERIALS AND METHODS

A total of about 1204 accession of maize landraces were collected from the villages of 9 districts of Manipur viz Imphal West, Imphal East, Bishnupur, Churachandpur, Tamenglong, Chandel, Thoubal, Senapati and Urkhrul where maize has grown extensively to tap the maximum genetic diversity. Each and every accession collected was assigned a passport data. The passport data comprised of Collection number, Collecting institute, Acquisition date, Farmer name, Province, Latitude of the collection site, Longitude of the collection site, Altitude of the collection site, Local Name given by farmer, Collection source. In Kharif 2014 the collected accession from all 9 districts of Manipur was categorised into similar group w.r.t. Kernel shape, kernel color, kernel row arrangement and Local name to avoid the duplicate sample. From each group a single accession was selected representing all the similar accession, the selected accession were planted and data were recorded from10 representative plant and was self pollinated in the experimental field of Plant Breeding and Genetics Department for preliminary evaluation. From the data recorded their mean was calculated, those accession which are very similar in relation to the quantitative as well as qualitative character are considered as duplicate samples and eliminated. Only 42 accessions were selected for further characterization and evaluation. In Kharif 2015, the 42 selected true maize landraces were shown in Randomised block design with 3replication, plot size having (4 X 2) m2 and spacing of 60 cm X 30 cm. Agronomic practices which is required for raising good crops was followed. The observations were recorded from ten randomly selected plants in each plots in each replication on 100Kernel Weight(g), Ear Height(cm), Growing degree unit (GDU) to Silking, Growing degree unit (GDU) to tasseling, No. of leaves above the uppermost ear, Plant Height(cm), Prolificacy Index, Total carotenoid(µg/ g) and Kernel Color as per Descriptors for Maize, CIMMYT. The data collected were subjected to Analysis of Variance and Correlation analysis.

Carotenoid Analysis

The cobs were harvested manually when fully mature and dried under the shades to lower post-harvest grain moisture to 14 %. Kernels of each genotype were visually screened and uniform representative kernels about 30 are randomly selected for each germplasm for 3 replications separately. The 30 representative kernels for each and every replication were grinded to fine flour using grinder machine by controlling the elevated temperature during grinding. The grinded maize flour was sieved through the fine muslin cloth. The sieved fine flour for each genotype was packed in water proof butter paper bags and kept at 4°C before the processing for extraction of carotenoids.

Finely powdered dry maize Kernel material (3 g) was hydrated at room temperature in water (10 ml for 30 min) followed by addition of ice-cold acetone (20 ml for 15 min). Carotenoids were extracted by grinding the mixture in a pestle and mortar with (50 ml) ice-cold acetone. The residue was filtered in a Buchannan funnel equipped with filter paper (Whatman #2, Schleicher and Schuel, UK). The residue was returned to mortar and the procedure was repeated until the residue become nearly colorless. One-third of the filtrate was transferred to a separating funnel containing 20 ml petroleum ether, to which 300 ml distilled water was added. After aqueous and organic layers were separated, the aqueous layer was discarded. The procedure was repeated for the remaining filtrate; the organic phase was washed three times with 200 ml of water and passed through anhydrous sodium sulfate into a round bottom flask (15 g). The organic phase was made up of 25ml with petroleum ether and carotenoids are estimated.

Estimation of Total Carotenoids

Estimation of total carotenoids was done by measuring the absorbance at 450 nm Using UV-Spectrophotometer after making up with petroleum ether to 20 ml; Petroleum ether was used as a blank. The carotenoid content in 3g of the sample was calculated by the method of Delia et al., 2004.

$$\text{Total carotenoid content } (\mu g/g) = \frac{\text{A total X Volume of extract (ml) X } 10^4}{\text{A1\% /1cm X sample wt (g)}}$$

Where,

A total = absorbance

Volume = total volume of extract and

A1% /1cm = absorption coefficient of 2500, which is recommended for mixtures.

Each sample was analyst three times and the data were subjected to statistical analysis using SAS 9.3.

Statistical Analysis

The statistical packaged, SAS 9.3 version available at CAU, Imphal was exclusively used for data analysis purpose. Some of the commonly used statistical tools namely the analysis of variance (ANOVA), Pearson's correlation analysis etc were applied in the 8 quantitative traits *viz* 100Kernel Weight(g), Ear Height(cm), Growing degree unit(GDU) to Silking, Growing degree unit (GDU) to tasseling, No. of leaves above the uppermost ear, Plant Height(cm), Prolificacy Index and Total carotenoid (µg/ g) and separate Pearson's simple correlation as well as Spearman's correlation were calculated between total carotenoid and kernel color. For the correlation analysis between total carotenoid and kernel color Pearson's simple correlation of 42 representative landraces were grouped according to Kernel color Mishra and Singh(2010) such as white(0), dark yellow(1), yellow(2), light yellow(3), orange(4), light orange(5), light red(6), dark red(7), black(8), violet(9), violet black(10), light red with white cap(11) and red with white cap(12). For spearman's ranks correlation analysis the qualitative data (kernel color) were ranked from orange(13), light orange(12), dark yellow(11), yellow(10), light yellow(9), dark red(8), light red(7), red with white cap(6), light red with white cap(5), violet(4), violet-black(3), black(2) and white(1). The ranking was done from orange to white according to highest to lowest total carotenoid concentration according to Tiwari *et al*.(2012).

RESULTS AND DISCUSSIONS

The data on the 8 quantitative traits *viz* 100Kernel Weight (g), Ear Height (cm), Growing degree unit (GDU) to Silking, Growing degree unit (GDU) to tasseling, No. of leaves above the uppermost ear, Plant Height (cm), Prolificacy Index and Total carotenoid (µg/ g) were analyzed in Randomized block design (RBD) to test the statistical validity of variance among different landraces. Extensive phenotypic variations were observed for all the measured quantitative traits in these maize landraces, as shown by the descriptive statistics in Table 1.

Table1: Descriptive Statistics for Eight Characters in Maize Landraces of Manipur, India

Variable	Mean	Std. Dev	Minimum	Maximum
100 Kernel Weight(g)	25.26	5.45	12.09	42.31
Ear Height(cm)	127.17	32.14	58.70	177.96
GDU to Silking	1016.62	23.55	992.85	1125.48
GDU to tasseling	962.58	35.29	870.22	1095.85
No. of leaves above the upper most ear	6.63	0.56	4.83	7.83
Plant Height(cm)	218.24	45.48	97.22	281.13
Prolificacy Index	1.51	0.36	1.10	3.10
Total carotenoid(µg/ g)	19.78	6.61	11.48	41.40

Analysis of variance revealed significance difference for all the character understudied which indicates that 42 landraces used in the investigation were genetically different for 8 agronomic traits (Table 2).

Table 2: Analysis of Variance for Maize Landraces of Manipur, India

Source	d. f.	Mean Sum of Squares			
		100 Kernel weight	Ear Height (cm)	GDU to Female Flowering	GDU to Male Flowering
Replication	2	39.12	21.74	0.41	0.73
Treatment	41	162.73**	358.14**	27.60**	7.89**

Source	d. f.	Mean Sum of Squares			
		Number of Leaves above the Uppermost Ear Including Ear Leaf	Plant Height (cm)	Prolificacy Index	Total Carotenoid
Replication	2	12.33	22.28	44.27	3.48
Treatment	41	8.09**	272.58**	23.78**	56.48**

The quantitative estimation revealed the wide range of carotenoids content among 42 landraces (Table 3).

Table 3: Total Carotenoids and Kernel Color of Maize Landraces of Manipur, India

SI No.	Accession Code	Kernel Color	Total Carotenoid (µg/ g)	SI No.	Accession Code	Kernel Color	Total Carotenoid (µg/ g)
1	CAUCH 39	Orange	18.84	22	CAUCH 57	Violet black	18.30
2	CAUCH 97	Light orange	33.09	23	CAUCH 69	Light yellow	13.15
3	CAUCH 73	Dark red	20.36	24	CAUCH 1007	Light yellow	17.47
4	CAUCH 93	Light yellow	15.57	25	CAUCH 66	Orange	41.40
5	CAUCH 1004	Yellow	14.38	26	CAUCH 78	Light yellow	36.25
6	CAUCH 1005	Light yellow	21.17	27	CAUCH 1000	Yellow	14.83
7	CAUCH 92	White	19.10	28	CAUCH 82	Light orange	30.31
8	CAUCH 1003	Violet	24.34	29	CAUCH 118	Light yellow	17.73
9	CAUCH 15	Light yellow	16.06	30	CAUCH 173	Dark yellow	20.50
10	CAUCH 36	Light yellow	16.23	31	CAUCH 35	Red with white cap	17.82
11	CAUCH 71	Light yellow	15.74	32	CAUCH 38	Light yellow	26.79
12	CAUCH 95	Light orange	23.22	33	CAUCH 50	Light red with white cap	19.58
13	CAUCH 321	Light yellow	11.48	34	CAUCH 90	Dark red	12.54
14	CAUCH 27	Light orange	19.92	35	CAUCH 76	Light yellow	13.90
15	CAUCH 17	Light red	17.02	36	CAUCH 117	Orange	17.64
16	CAUCH 70	Light yellow	12.89	37	CAUCH 85	Light yellow	15.75
17	CAUCH 61	Light yellow	21.78	38	CAUCH 21	Light yellow	15.05
18	CAUCH 87	Light yellow	25.96	39	CAUCH 28	Yellow	14.69
19	CAUCH 81	Light orange	19.92	40	CAUCH 166	Orange	32.38
20	CAUCH 1006	White	24.02	41	CAUCH 220	Light yellow	14.25
21	CAUCH 19	Light yellow	22.48	42	CAUCH 1008	Light violet	12.71

The mean value for total carotenoids content was found to vary from minimum of 11.48 µg/ g in landraces CAUCH 321 to the maximum of 41.40 µg/ g in CAUCH 66 with the population mean of 19.78 µg/ g. Among the 42 landraces orange kernel color CAUCH 66 recorded highest total carotenoids (41.40 µg/ g), CAUCH 321(11.48 µg/ g) recorded as lowest which have light yellow kernel color. The kernel color white i.e. CAUCH 92and CAUCH 1006 recorded 19.10 µg/ g, 24.02 µg/ g respectively. However, there is a varying degree of total carotenoids content even in orange kernel color CAUCH 39 (18.84µg/ g), CAUCH 117(17.64 µg/ g) and CAUCH 166(32.38 µg/ g). The varying degree of total carotenoid comparing to kernel color is due to the involvement of several enzymes other than phytoene synthase which gives yellow color and controls the rate-limiting step in the carotenoid biosynthetic pathway. Besides phytoene synthase, other enzymes such as phytoene desaturase (*pds*), zeta carotene desaturase (*zds*), lycopene beta cyclase (lcyB), lycopene epsilon cyclase (lcyE) and beta carotene hydroxylase 1 (crt RB1) are involved in important catalytic steps of the pathway(Harjes *et al*, 2008 and Yan *et al*, 2010).

The correlation coefficient for both the Pearson's correlation and Spearman's rank correlation showed that the total carotenoid content and kernel color are independent (Harjes *et al*, 2008. Mishra and Singh, 2010). Pearson's correlation analysis which is mainly used in quantitative data can also be used in qualitative data after converting them with appropriate scores (Table 4).

Table 4: Correlation Between Total Carotenoids and Kernel Color of Maize Landraces of Manipur, India

Pearson Correlation Coefficient					Spearman Rank Correlation Coefficient				
	Total Carotenoid	Kernel Color	t Value	Table Value		Total Carotenoid	Kernel Color	t Value	Table Value
Total carotenoid	1	0.07(ns)	0.42	2.02	Total carotenoid	1	0.29(ns)	1.99	2.02
Kernel Color		1			Kernel Color		1		

Pearson's correlation coefficients of the 8 quantitative characters are presented in Table 5. The result shows that there was the strong positive correlation between 100 kernel weight with plant height where, r= 0.41* Elezi *et al*. (2013). Ear height with plant height where r=0.78* and a strong positive correlation between GDU to silking with GDU to tasseling where, r=0.82*. A negative correlation between Ear height and Total carotenoid was found with r=-0.39*. For the other features, there was no significant correlation. The correlation analysis revealed non- significant relation between kernel color and total carotenoid.

Table 5: Phenotypic Correlation Coefficients Among Eight Characters in Maize Landraces of Manipur, India

Pearson's Correlation Coefficient, N=42								
	KW	**EH**	**GDUS**	**GDUT**	**NLAUE**	**PH**	**PI**	**TCRT**
KW	1.00	0.24	0.01	-0.01	0.29	0.41*	0.08	0.09
EH		1.00	0.22	0.12	0.10	0.78*	-0.16	-0.39*
GDUS			1.00	0.82*	0.12	-0.04	0.11	-0.21
GDUT				1.00	0.01	-0.07	0.05	-0.18
NLAUE					1.00	0.30	0.14	0.20
PH						1.00	-0.21	-0.31
PI							1.00	-0.08
TCRT								1.00

KW= Kernel Weight; EH= Ear Height; GDUS= GDU to Silking; GDUT= GDU to tasseling; NLAUE= No. of leaves above the uppermost ear; PH= Plant Height; PI= Prolificacy Index and TCRT= Total carotenoid

The significance of the correlation coefficients at the 5% or 1% level is indicated by * or**respectively and ns indicates non- significant.

CONCLUSIONS

The present investigation revealed the presence of considerable genetic variation for all the character understudied in the selected maize landraces of Manipur signifying that there is a scope of genetic improvement for this important trait. The landraces which have the highest total carotenoid needs to be further profiled for the components of carotenoids and those landraces have to be given preference in growing and distribution to the people to ease Vitamin A deficiency in the region. Although carotenoids are the different coloring pigments in plants it does not have any significance to the total carotenoids which have the Vitamin A activity, therefore merely selecting maize based on kernel color will be a non-scientific effort.

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